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Tamm-Horsfall protein antibody in patients with end-stage kidney disease

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TAMM-HORSFALL PROTEIN ANTIBODY

IN PATIENTS WITH END-STAGE KIDNEY DISEASE

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ABSTRACT

Antibody to Tamm-Horsfall protein (THP) was measured using a radioimmunoassay in 45 patients on chronic hemodialysis. Of these 45 patients, 13 had renal failure secondary to polycystic kidney disease, 14 had failure secondary to glomerular diseases, nine had failure secondary to diabetes, three had failure secondary to interstitial nephritis, three had failure secondary to chronic pyelonephritis, two had failure secondary to multiple myeloma, and one had failure secondary to urinary tract obstruction. Results from these 45 patients were compared to levels of antibody measured in sera from 10 healthy controls. In no group was the mean titer significantly different from the mean of the control group. Four patients had significantly elevated titers of antibody but there were no correlating features among them.

Observations during the study revealed that heparinized samples of blood, following cell removal, had lower titers of antibody to THP than did non-heparinized samples from the same patient. Further investigation of this finding, using other anticoagulants, lead to the suggestion that clotting factors were the cause of the observed interference.

INTRODUCTION

Since its discovery and characterization by Tamm and Horsfall (1) the importance of the 80,000 molecular weight glycoprotein which carries their name remains a mystery. Only the cells lining the ascending loop of Henle and distal convoluted tubule synthesize the protein, which is secreted into the tubular lumen (2,3). No physiological role for the protein is known although the original investigators noted it inhibited hemagglutination caused by influenza, mumps and Newcastle disease virus (1), but had no effect on bacteria (4). Approximately 50 mg of Tamm-Horsfall protein (THP) is excreted per day in human urine, and in the presence of low pH or high salt concentration in the urine, the protein aggregates and forms casts (4-6).

Recent work implicates THP in various pathological processes affecting the kidney, including calculi formation (7), and other precipitates which may be a factor in the pathogenesis of acute renal failure by causing microscopic tubular obstruction (8).

In addition, THP and the immunologic response induced by it are implicated in many interstitial diseases of the kidney. Rats immunized to homologous THP develop tubulo-interstitial nephritis (9). In humans, antibody to THP has been described in patients with reflux nephropathy (10), with urinary tract obstruction (UTO) and urinary tract infection (UTI) (11), and pyelonephritis (12). Intrarenal deposits of THP have been described in medullary cystic disease (13), chronic pyelonephritis, and hydronephrosis with suggestions that THP may be involved in the pathogenesis of these diseases (13,14).

One theory which explains the potentially pathogenic role of THP suggests that THP is normally not seen by the immune system and that with obstruction or reflux, urine containing THP flows retrograde into renal parenchymal lymphatics and veins (10,15,16), thus rendering this sequestered protein immunogenic. Indeed, urine does reflux into lymphatics and renal veins in patients with UTO and vesico-ureteral reflux (VUR) (17,18). Also, tubulo-interstitial nephritis has been produced in animals immunized with THP (9).

If this same process of immunization occurs in man, patients with VUR or UTO might become immunized to their own THP and a useful marker of early disease would be titers of the induced anti-THP antibody. Conceivably these titers would be elevated before irreversible renal damage occurred and would provide a simple, non-invasive method for detection of early disease. Recent work in this laboratory (11) showed significant elevations of THP antibody in 5/15 patients with UTO and UTI when compared to healthy controls. Others have shown elevated titers of antibody in school age girls with symptomatic "upper" UTI (12). However, there is no evidence for the presence of anti-THP antibody in patients with later or end-stage kidney disease.

The natural history of VUR can be insidious. In one study (19) some patients had VUR undiagnosed until renal failure occurred. These patients had parenchymal changes in their kidneys which were characteristic of chronic pyelonephritis (CPN). Others have shown that severe VUR plus UTI lead to renal scarring and fibrosis (20).

Since VUR and UTO can lead to CPN and end-stage renal disease, and since increased anti-THP antibody have been demonstrated in school age girls with

acute pyelonephritis (12) and in patients with UTO and infection (11), it seemed important to determine anti-THP antibody titers in patients with CPN, UTO and interstitial nephritis (IN). This might help to define the role, if any, of THP antibody in the pathogenesis of these diseases.

The current study evaluates patients with end-stage renal disease on maintenance hemodialysis, with diagnoses of UTO, IN, and CPN, and compares them with patients with other causes of end-stage renal disease including polycystic kidney disease (PCKD), glomerulonephritis (GN), and diabetic nephropathy (DN), as well as healthy control subjects.

METHODS

Patient Selection

A total of 45 chronic hemodialysis patients at Yale-New Haven Hospital and the West Haven Veterans Administration Hospital participated in this study after giving informed consent. All of these patients had a diagnosis for their renal failure based on clinical, radiological or histological evidence. This information, along with information about UTI, current and past medications and length of time on dialysis, was obtained from the patient's hospital record.

Patients were placed into groups based upon the etiology of their renal failure. These groups included: (1) Thirteen patients with PCKD where the diagnosis was made by intravenous pyelography or laparotomy. (2) Nine patients with DN, diagnosis made on clinical evidence of diabetes mellitus and sequellae including cataracts and retinopathy in eight of nine, and one patient with history of diabetes and a biopsy which showed interstitial nephritis. (3) Fourteen patients with glomerulonephritis (GN), eleven of fourteen had biopsies, showing membranoproliferative GN in three, membranous GN in two, chronic progressive hypo-complementemic GN in one, and chronic GN (CGN) in five. In the remaining three patients without biopsy, the diagnosis of CGN was based on a history of acute GN which progressed to renal failure in one, a history of beta hemolytic streptococcal infection followed by hematuria, albuminuria, and progressive renal failure in another, and a history of skin infections leading to progressive renal failure in the third. (4) Six patients with the diagnosis of CPN or IN, whose diagnoses were based on a history of phenacetin abuse in one, neomycin induced deafness and renal failure in one, biopsy in two, and by renal scan and pyelography in the other two. (5) One patient with

UTO diagnosed with IVP, and (6) two patients with multiple myeloma(secretory type) who developed renal failure with progression of their disease.

Blood Collection

Blood was obtained from each patient for determination of anti-THP antibody titers. In order to evaluate the effect of dialysis on titers of anti-THP antibody, 5 ml samples of blood were obtained from participants pre- and post dialysis for later comparison. The samples from Yale-New Haven Hospital were collected in plain vacutainers and allowed to clot at room temperature for one hour before serum was removed. The samples from the West Haven Veterans Administration Hospital were collected in heparinized vacutainers (approximately 120U Hep) and the plasma removed immediately. Sodium azide was added to each specimen for bacteriostasis.

All patients received heparin during dialysis, an initial bolus of 5000 U followed by three hourly 1000 U doses until dialysis ended after four hours. Heparin antagonists were not given to any of the patients.

Urine

Clean catch urine specimens were obtained for culture from all patients who were not anuric. Quantitative urine cultures were performed by the standard pour plate technique. Bacteria recovered were identified by the scheme of Schaub and Foley (21).

THP Antibody

Serum antibody titers to THP were determined by a radioimmunoassay initially developed in this laboratory and described in detail in previous publications (11,22). The assay depends on binding specific antibody to a solid phase antigen (THP adsorbed to a microtiter plate). The subsequent

identification of bound antibody was achieved by adding I^{125} labeled Protein A which binds to the Fc region of IgG.

Serial dilutions of rabbit sera containing known amounts of specific antibody were assayed simultaneously with the patient samples to obtain a standard curve for conversion of counts per minute to ng antibody/ml.

Heparin Effect

Preliminary results indicated that the heparinized samples gave consistently lower anti-THP titers than unheparinized samples. Further studies were done to elucidate the effect of heparin on the assay.

Heparinized and non-heparinized samples from medical student "volunteers" were obtained to evaluate the effect of heparin on freshly collected samples. The non-heparinized samples were allowed to clot for one hour and the serum was then removed. The heparinized samples were immediately centrifuged and plasma removed. All samples received sodium azide as a preservative.

To evaluate the effect heparin might have directly on antigen-antibody binding in the assay, heparin was added to serum from a rabbit immunized to human THP in amounts approximately equal to 5x, 10x, 50x, and 100x the calculated concentration of heparin in patients' serum immediately following dialysis. The amount of heparin added was calculated by approximating the heparin T ½ in serum at 60 minutes (23) for the amounts used. Samples containing rabbit serum and heparin were then evaluated for anti-THP activity and compared to serum from the same rabbit which received no heparin.

In order to determine whether heparin exerted a specific effect, or if the inhibition observed was a result of some other plasma component, i.e. fibrinogen, blood samples anticoagulated with EDTA and Na citrate were compared to samples which were heparinized and samples which were not anticoagulated. Four samples of blood were taken from each of ten volunteers;

one sample without anticoagulent, one with heparin, one with EDTA, and one with Na citrate. As a further study to evaluate the effect of fibrinogen on the assay, samples collected with EDTA and citrate were defribinated by adding back $CaCl_2$. This was done by incubating samples and $CaCl_2$ (final concentration in samples 0.025M) for one hour at 37°C and then at 4°C for twelve hours (24). The resulting clot was removed and the samples assayed.

Statistical Methods

Patients with antibody titers greater than the mean level of healthy control subjects plus two standard deviations were defined as significantly elevated titers. Independent groups were compared using T-statistics for independent data, heparin vs. serum comparisons were made using the paired T test.

RESULTS

Patients with End-Stage Kidney Disease

Figure 1 displays the antibody titer of patients grouped by diagnosis and includes controls. The mean anti-THP antibody titer in ten control subjects was 1050 ng/ml and the mean plus two standard deviations was 1920 ng/ml. Any titer above this value was considered significant. Sera from the thirteen patients with PCKD gave a mean anti-THP antibody titer of 710 ng/ml ± 201 ng/ml with no values above the level of significance. The mean value of anti-THP antibody in sera from fourteen patients with GN was 1190 ng/ml ± 1040 ng/ml which included two patients with significantly elevated titers of 5130 ng/ml and 4440 ng/ml. Sera from nine patients with DN gave a mean titer of 1260 ng/ml ± 740 ng/ml and included one patient with a titer of 4330 ng/ml. Sera from the three patients with CPN gave a mean of 1480 ng/ml ± 976 ng/ml and none were elevated above normal values. Sera from the three patients with IN gave a mean of 1580 ng/ml ± 290 ng/ml including a significant titer in one patient, 2050 ng/ml. Sera from the two patients with multiple myeloma gave a mean titer of 340 ng/ml and were the lowest for any single diagnostic group. The serum from the one patient with UTO gave a titer of 1090 ng/ml.

The mean values for anti-THP antibody for each of the groups were not significantly different from mean values for healthy subjects. The highest mean 1580 ng/ml was seen in patients with IN, followed by CPN 1480 ng/ml, DN 1260 ng/ml, GN 1190 ng/ml, UTO 1090 ng/ml, PCKD 710 ng/ml, and MM 340 ng/ml.

Evaluation of urine culture results from each patient revealed a total of twelve patients with significant levels of urinary bacteria. Sera from the four patients infected with E. coli had a mean titer of 1640 ng/ml ± 1280 ng/ml. Sera from the eight other patients with infected urine had a mean

anti-THP antibody titer of 876 ng/ml ± 257 ng/ml. Sera from the eleven patients with sterile urine had a mean of 850 ng/ml ± 376 ng/ml and the twenty-two patients who were anuric had a mean titer of 1180 ng/ml ± 775 ng/ml. Individual results appear in Figure 2. None of the groups were significantly different from the control group. Of the four patients with significantly elevated titers, one had an E. coli UTI and the other three were anuric.

Figure 3 presents each patient's serum titer of anti-THP antibody as a function of the number of months he/she had been on dialysis at the time of the study. Figures 4-7 show the same function for each diagnostic group, Figure 4 PCKD, Figure 5 DN, Figure 6 GN, Figure 7 CPN and IN. Of patients dialyzed twenty months or less, 1/11 had significantly elevated titers. Of patients dialyzed between twenty and forty months, 3/14 had elevated titers. None of the other nineteen patients had elevated titers. For each group there is no trend except for CPN-IN, which suggests a downward trend in activity.

Heparin vs. Non-Heparin

Pre-dialysis samples (non-heparinized at Yale-New Haven Hospital, heparinized at the West Haven Veterans Administration Hospital) and post-dialysis samples (heparinized during dialysis at both hospitals) were compared by hospital. There is a suggestion of significant difference between means of pre- (1237 ng/ml) and post- (1081 ng/ml) samples at Yale-New Haven Hospital ($p=.03$) while there is no significant difference between pre- (637 ng/ml) and post- (666 ng/ml) samples at the West Haven Veterans Administration Hospital ($p = 0.64$).

Figure 8 presents results of serum and plasma anti-THP titers in ten volunteers expressed in cpm. Each volunteer gave two samples of blood, one

heparinized and one not heparinized. The mean antibody titer for non-heparinized samples was 7530 cpm ± 4040 cpm and the mean antibody titer in heparinized samples was 5600 cpm ± 2780 cpm. These means are significantly different ($p = 0.003$).

Rabbit Serum

Figure 9 shows results of adding heparin to rabbit serum, making a final heparin concentration of 5x - 100x the concentration of heparin in patients at the end of dialysis. Anti-THP activity remains the same regardless of heparin concentration.

Other Anti-Coagulants

The results of adding anti-coagulants to freshly drawn samples are presented in Table III. Ten volunteers contributed four samples each. There is a significant difference between control sera and sodium citrate ($p=0.003$), EDTA ($p=0.004$), and heparin ($p=0.01$). No significant difference between control and control + calcium ($p=0.1$), citrate + calcium ($p=0.02$), and EDTA + calcium ($p=0.2$). However, there is a significant difference between control and heparin + calcium ($p=0.01$).

DISCUSSION

The objective in these studies was to determine if a new assay which detects small amounts of anti-THP antibody (500 ng/ml) (11) in healthy patients, might detect elevated titers of antibody in patients with various forms of end-stage kidney disease. A working hypothesis for this study suggested that titers would be elevated in patients with UTO, CPN, or IN, because anti-THP might be involved in the pathogenisis of these diseases. Although the present work indicates that none of the groups studied had mean titers significantly different from controls, it is interesting that the group of patients with CPN and IN had the highest anti-THP antibody titers.

Although no general trend is apparent for decreasing amounts of anti-THP as a function of number of months on dialysis, it is possible that disease in these patients is quiescent, with decreased amounts of antigen available for immune reaction, and subsequently decreased immune response and measurable antibody.

Previous attempts to identify antibody to kidney tissue in patients with pyelonephritis and chronic nephritis using complement fixation (25), latex agglutination (26), and hemagglutination (27), have been unsuccessful. However, those assays were less sensitive than the present one and they could not identify circulating antibody in normal controls. Using this assay, Marier et al (11) demonstrated anti-THP in normal controls and significantly elevated titers in patients with VUR and UTI. Hanson (12) also observed elevated anti-THP titers in 9/10 school girls with upper UTI using an enzyme linked immuno-absorbant assay.

In the present study, four of the forty-five patients studied had significantly elevated antibody titers, but they did not share any other unifying

characteristic. Two patients with GN had elevated titers, one of whom also had an E. coli UTI. One patient with DN had elevated titers and was anuric, and another anuric patient with elevated titers had kidney failure based on long standing IN.

Several hypotheses exist to explain previously observed (11,12) elevated anti-THP antibody titers in patients with kidney disease. One hypothesis suggests the presence of common immunological determinants shared by bacteria and normal kidney tissue so that infection with one of these bacterial types leads to an immune response to the invading bacteria and also to the shared determinant in normal kidney tissue. If these kidney determinants were on tubular cells, then pyelonephritis might occur as a result of immunologic injury. Holmgren et al (28) using immunodiffusion techniques have shown that there are common determinants between normal kidney tissue and certain strains of E. coli. Further evidence supporting this hypothesis is presented by Kalmanson and Guze (29) who observed that healthy rats parabiosed to rats recovering from E. coli pyelonephritis developed histologic evidence of interstitial nephritis which resembled chronic pyelonephritis. This experiment does not prove the theory of shared determinants because infectious damage to the tubules might release kidney tubule antigen which in turn might be recognized as foreign, thus propagating a second and separate immune response. These observations suggest a second hypothesis: tubular injury of any type leads to release of antigen, with a subsequent, secondary immune reaction involving kidney determinants (30). A third mechanism by which normal kidney proteins might be recognized as foreign following UTI is similar to the mechanism by which methicillin associated interstitial nephritis occurs (31), where methicillin is immunogenic when combined with a tubular protein leading to an immune response to both the protein and

the protein plus methicillin. Kidney proteins might be carriers of bacterial hapten, thus initiating an immune response to normal kidney tissue following infection.

Finally, infectious damage to tubules need not be the only mechanism leading to an immune reaction in the kidney. Any insult to the tubules (including toxins, obstruction or ischemia) might lead to release of immunogenic proteins and might initiate immunologically mediated renal disease.

McCluskey and Colvin (32) in their review of immunological aspects of renal tubular and interstitial disease suggest a mechanism by which patients with GN might have elevated titers of anti-kidney antibody. Many types of GN including membranoproliferative GN and rapidly progressive GN have associated tubulointerstitial deposits of immunoglobulin (33). Possibly the glomerular disease damages the tubule leading to release of antigen, and an immune response.

The role of THP in pathogenesis of renal disease is not definitively determined, yet it need not be actively involved in the pathological process to be valuable as a serological marker of active renal pathology.

Heparin

Results obtained from patients at Yale-New Haven Hospital and the West Haven Veterans Administration Hospital indicate that heparin inhibits the measurement of anti-THP antibody. These results also showed a significant difference between anti-THP measured in heparinized and non-heparinized samples from the same person with decreased activity in heparinized samples. Adding heparin directly to serum demonstrates that heparin does not directly interfer with antigen-antibody binding, but must act through some substance present in plasma but not present in serum, such

as fibrinogen or heparin activated anti-thrombin III. Heparin exists as a heterogenous assortment of polymeric chains, and each chain length may have different physiological characteristics (34). It prevents coagulation of blood by binding to and activating anti-thrombin III, which then binds to and inhibits certain activated factors of the coagulation cascade (XIa (35), IXa (36), Xa (37), and thrombin (38)).

One other possible source of interference with the RIA is fibrinogen. It might interfer by non-specifically binding to the solid phase antigen or to the added antibody, leading to a decrease in anti-THP titers.

The further experiments with anti-coagulants including EDTA and sodium citrate, show that the inhibition initially observed in heparinized blood also appears in blood anti-coagulated with EDTA and citrate. Levels of antibody (in cpm) in specimens prepared with these two chelators of calcium, return to near control values after defibrinization with $CaCl_2$. These results suggest fibrinogen as the serum protein which intergers with the RIA.

The results presented here show that nearly all patients with end-stage kidney disease on dialysis do not have elevated titers of antibody when compared to normal controls. Larger study groups may be necessary in order to determine whether significant differences exist.

Dialysis patients with end-stage kidney disease probably do not have an active pathological process in their kidneys. The normal anti-THP antibody titers in the patients studied here are the expected results, which reflects a return to normal levels of antibody if the hypothesis of THP mediated disease is correct. Future study of patients with active kidney disease, especially IN and CPN, may shed more light on the pathogenic role of THP in these diseases and may define more clearly the meaning of elevated anti-THP antibody titers already observed in certain patients (11,12).

During the course of the present study, it was noted that antibody titers measured by radioimmunoassay were lower in heparinized as compared with non-heparinized blood. The decrease in antibody titer was related to interference in the assay by fibrinogen rather than to any specific effect heparin might have on antigen-antibody interactions.

REFERENCES

1. Tamm, I., Horsfall, F.L., Jr. Characterization and separation of an inhibitor of viral haemagglutination present in urine. Proc. Soc. Exp. Biol. Med. 74:108-114, 1950.

2. Schrenk, E.A., Schwartz, R.H., Lewis, R.A. Tamm-Horsfall protein. 1. Localization in the kidney. Lab. Invest. 25:92-95, 1971.

3. Pollack, V.E., Arbel, C. The distribution of Tamm-Horsfall mucoprotein (uromucoid) in the human nephron. Nephron 6:667-672, 1969.

4. McQueen, E.G., Engel, G.B. Factors determining the aggregation of urinary mucoprotein. J. Clin. Path. 19:392, 1966.

5. McKenzie, J.K., Patel, T., McQueen, E.G. The excretion rate of Tamm-Horsfall protein in normals and in patients with renal disease. Aust. Ann. Med. 13:32, 1964.

6. McQueen, E.G. The nature of urinary casts. J. Clin. Path. 15:367, 1962.

7. Grant, A.M.S., Baker, L.R.I., Neuberger, A. Urinary Tamm-Horsfall protein in certain kidney disease and its content in renal and bladder calculi. Clin. Sci. 44:377-384, 1973.

8. Patel, R., McKenzie, J.K., McQueen, E.G. Tamm-Horsfall urinary mucoprotein and tubular obstruction by casts in acute renal failure. Lancet 1:457, 1964.

9. Hoyer, J.R. Autoimmune tubulointerstitial nephritis induced in rats by immunization with rat Tamm-Horsfall protein. In Abstracts of the Ninth Annual Meeting of the American Society of Nephrology. Charles B. Slack, Thorofare, NJ, 1976, p. 58.

10. Hodson, C.J., Maling, T.M.J., McManamon, P.J., Lewis, M.G. Reflux nephropathy. Kidney Int. 8:S50-S58, 1975.

11. Marier, R., Fong, E., Jansen, M., Hodson, J., Richards, F., Andriole, V.T. Tamm-Horsfall protein antibody in urinary tract obstruction and vesicoureteral reflux. J. Infect. Dis. 138:781-790, 1978.

12. Hanson, L.A., Fasth, A., Jodal, U. Auto antibodies to Tamm-Horsfall protein, a tool for diagnosing the level of urinary tract infections. Lancet Jan. 31, 1976, p. 226.

13. Vernier, R.L., Resnick, J. Medullary cystic disease: the possible role of Tamm-Horsfall protein. Kidney Int. 9:450, 1976.

14. Zager, R.A., Cotran, R.S., Hoyer, J.R. Pathologic localization of Tamm-Horsfall protein in interstitial deposits in renal disease. Lab. Invest. 38:52, 1978.

15. Bakshandeh, K., Lynne, C., Carrion, H. Vesicoureteral reflux and end stage renal disease. J. Urol. 116:557, 1976.

16. Rolleston, G.L., Maling, T.M.J., Hodson, C.J. Intrarenal reflux and the scarred kidney. Arch. Dis. Child. 49:531-539, 1974.

17. Bell, R.D., Ornitz, R.D., Trautman, R., Anderson, R., Keyl, J. The significance of the renal pelvis and intrarenal veins in renal lymph

formation. Invest. Urol. 9:149, 1971.

18. Hodson, C.J., Edwards, D. Chronic pyelonephritis and vesicoureteral reflux. Clin. Radiol. 11:219-231, 1960.

19. Salvatiena, O., Jr., Kountz, S.L., Belzer, F.D. Primary vesicoureteral reflux and end stage renal disease. JAMA 226:1454, 1973.

20. Lenagham, D., Whitaker, J.G., Jensen, F., Stephens, F.D. The natural history of reflux and long term effects of reflux on the kidney. J. Urol. 115:728, 1976.

21. Schaub, I.G., Foley, M.K., Scott, G.G., Barley, W.R. Diagnostic Radiology. 5th Edition, Mosby, St. Louis, 1958.

22. Marier, R., Jansen, M., Andriole, V.T. A new method for measuring antibody using radiolabeled Protein A. Clin. Res. 26:518A, 1978.

23. Olsson, P., Lagergren, H., Ed, S. The elimination from plasma of intravenous heparin: an experimental study on dogs and humans. Acta Med Scand. 173:619-630, 1963.

24. Pitney, W.R., Dacie, J.V. A simple method of studying the generation of thrombin in recalcified plasma. J. Clin. Path. 6:9, 1953.

25. Mackay, I.R., Larkin, L. The significance of the presence in human serum of compliment fixing antibodies to human tissue antigens. Aust. Ann. Med. 251-258, 1958.

26. Kramer, N.C., Watt, M.F., Howe, J.H., Parrish, A.W. Circulating antihuman kidney antibodies in human renal disease. Am. J. Med. 30:39-45, 1961.

27. Kalmanson, G.U., Glassock, R.J., Harwick, H.J., Guze, L.B. Cellular immunity in experimental pyelonephritis. Kidney Int. 8:S35-S43, 1975.

28. Holmgren, J., Hanson, L.A., Holm, S.E., Kaijser, B. An antigenic relationship between kidney and certain E. coli strains. Int. Arch. Allergy Appl. Immunol. 41:463-474, 1971.

29. Kalmanson, G.U., Guze, L.B. Pyelonephritis. An attempt to demonstrate anti-kidney antibody in sera of patients with chronic bacteriuria. Am. J. Med. Sci. 246:532-536, 1963.

30. Losse, H., Intorp, H.W., Lison, A.E., Funke, C. Evidence of an autoimmune mechanism in pyelonephritis. Kidney Int. 8:S44-S49, 1975.

31. Border, W.A., Lehman, D.H., Egan, J.D., Sass, H.J., Glode, J.E., Wilson, E.B. Anti-tubular basement-membrane antibodies in methicillin-associated interstitial nephritis. N. Engl. J. Med. 291:381-389, 1978.

32. McCluskey, R.T., Colvin, R.B. Immunological aspects of renal tubular and interstitial diseases. Ann. Rev. Med. 29:191-203, 1978.

33. Lehman, D.H., Wilson, C.B., Dixon, F.J. Extraglomerular immunoglobulin deposits in human nephritis. Am. J. Med. 58:765-786, 1975.

34. Jaques, L.B., McDuffie, N.M. The chemical and anti-coagulant nature of heparin. Semin. Thromb. Hemo. 4:277, 1978.

35. Amir, T., Ratnoff, D.D., Pensky, J. Partial purification and some properties of a plasma inhibitor of activated plasma thromboplastin anticedent factor. J. Lab. Clin. Med. 80:786, 1972.

36. Rosenberg, J.S., McKenna, P.W., Rosenberg, R.D. Inhibition of human factor IXa by human antithrombin. J. Biol. Chem. 250:883-888, 1975.

37. Rosenberg, R.D. Function of heparin. In V.V. Kakker and D.P. Thomas (eds.) Heparin Chemistry and Chemical Usage. Academic Press, New York, 1976, p. 101-120.

TABLE 1

Clinical Data and Levels of Antibody to Tamm-Horsfall Protein (Pre- and Post- Dialysis Expressed in CPM) in 45 Patients with End-Stage Kidney Disease

Patient No. (Age, Sex)	Pre- cpm	Post- cpm	Δ%	Urine	Mos. on Dialysis	ng/ml
PCKD - YNHH						
1 (63, F)	2150	2650	+23	Enterobacter	39	477
2 (54, M)	2070	2820	+26	n.g.	34	455
3 (60, M)	2770	3090	+11	anuric	48	655
4 (49, M)	2920	3040	+4	anuric	29	700
5 (64, F)	3030	2460	-19	anuric	31	733
6 (60, F)	2390	2830	+19	anuric	48	544
7 (65, F)	5720	4380	-24	diptheroids	47	1625
8 (42, M)	3080	1850	-40	anuric	22	748
9 (36, F)	3890	3280	-16	diptheroids	14	1002
10 (20, M)	2260	3020	+33	anuric	37	508
WHVAH						
11 (59, M)	2390	2120	-11	E. coli	12	544
12 (52, M)	4030	3300	-18	anuric	106	1048
13 (58, M)	1630	2270	+39	anuric	60	337
n=13	x=2950	x=2837				x=721
	s=1080	s=642				s=341
DN - YNHH						
14 (60, F)	12500	9730	-22	anuric	31	4329
15 (37, M)	3930	3250	-17	n.g.	40	1015
16 (71, F)	4400	4330	-2	E. coli	38	1170
17 (49, F)	4210	3540	-16	anuric	10	1107
18 (59, F)	3010	2890	-4	anuric	96	727
19 (57, F)	1950	1650	-15	anuric	43	422
20 (49, F)	3330	4400	+32	anuric	19	825
WHVAH						
21 (64, M)	5140	5330	+4	anuric	119	1421
22 (61, M)	3670	4174	+14	diptheroids	6	932
n=9	x=4683	x=4370				x=1328
	s=3068	s=2270				
GN - YNHH						
23 (28, M)	14318	10040	-30	anuric	26	5132
24 (28, M)	3930	3310	+26	n.g.	9	1015
25 (32, M)	4030	3900	-3	anuric	117	1048
26 (26, M)	2460	4650	+90	anuric	59	565
27 (24, F)	3250	3620	+11	anuric	67	800
28 (35, F)	5360	5470	+2	anuric	56	1498
29 (47, M)	2890	1810	-37	n.g.	25	691
30 (19, F)	12750	11000	-14	E. coli	3	4437

TABLE 1
Continued

GN - WHVAH						
31 (59, M)	1810	2640	+45	S. aureus	7	384
32 (57, M)	1900	1410	-26	n.g.	1	408
33 (54, M)	4400	3690	-16	anuric	60	1170
34 (55, M)	1860	1750	-6	n.g.	64	398
35 (53, M)	3930	4460	+13	anuric	96	1015
36 (43, M)	1550	1350	-13	n.g.	88	316
n=14	x=4015 s=4470	x=4170 s=2980				x-1348
CPN - YNHH						
37 (59, M)	6020	5820	-3	n.g.	42	1733
38 (41, M)	5710	5350	-6	n.g.	49	1622
39 (34, M)	4190	3800	-10	anuric	48	1100
n=3	x=5300 s=976	x=4990 s=1060				x=1485
IN - YNHH						
40 (43, M)	6870	5390	-22	anuric	31	2045
41 (59, M)	5880	4300	-27	n.g.	77	1682
WHVAH						
42 (50, M)	4020	5000	-24	Enterococcus	124	1045
n=3	x=5590 s=1450	x=4900 s-552				x-1591
MM - YNHH						
43 (49, M)	1740	1000	-43	n.g.	21	366
WHVAH						
44 (51, M)	1540	1220	-21	Klebsiella	24	314
n=2	x=1640	x=1110				x=340
UTO - YNHH						
45 (21, M)	4170	3730	-11	Proteus	20	
n=45						

TABLE II

Levels of Antibody to Tamm-Horsfall Protein (expressed in CPM)

in Heparinized and Non-heparinized Samples Collected from Volunteers

Sample	Non-heparinized	Heparinized	%
KB	5140	3270	-36
GC	4340	4290	-1
BF	2800	2490	-11
ML	11,090	6430	-42
BS	5320	4730	-11
JW	5180	4930	-5
DB	9970	7400	-25
JB	16,310	12,280	-25
JK	6510	3980	-39
BS	8660	6030	-30
n=10	x=7530	x=5590	
	sd=4040	sd=2780	

TABLE III

Effect of Coagulation Factors on Levels of Antibody to

Tamm-Horsfall Protein (expressed in CPM) measured by this assay

Sample	CO	CI	ED	HE	CO+Ca	CI+Ca	ED+Ca	HE+Ca
AM	3570	2500	2280	2230	2380	2100	2340	1860
SD	2630	2230	2610	2400	2950	2135	2890	2120
TM	2710	2334	2400	2250	3150	2820	3070	2590
BC	3620	2590	2820	3090	3200	3190	3170	2820
RD	1830	1430	1510	1780	2130	1970	1990	1560
JS	2950	2300	2330	2660	3020	2560	2680	2410
SD	5120	3570	4300	3770	3770	3370	4130	4070
JW	3180	2060	2380	3090	3290	3360	4050	3070
SS	6290	4070	5310	4180	5200	4530	6480	5310
DB	7020	4040	4560	4230	5390	5410	4270	4120
n=10	x=3890	x=2720	x=3050	x=2970	x=3450	x=3140	x=3510	x=2990
	sd=1690	sd=894	sd=1230	sd=860	sd=1080	sd=1110	sd=1300	sd=1180

CO=control, CI=sodium citrate, ED=EDTA, HE=heparin, Ca=calcium chloride
x=mean, sd=standard deviation

FIGURE 1: Measurement of antibody to Tamm-Horsfall Protein (THP) in cpm and ng/ml in sera from patients with various types of end-stage kidney disease and control subjects. Bar graphs represent one standard deviation. PCKD = polycystic kidney disease, DN = diabetic nephropathy, GN = glomerulonephritis, CPN = chronic pyelonephritis, IN = interstitial nephritis, MM = multiple myeloma, UTO = urinary tract obstruction.

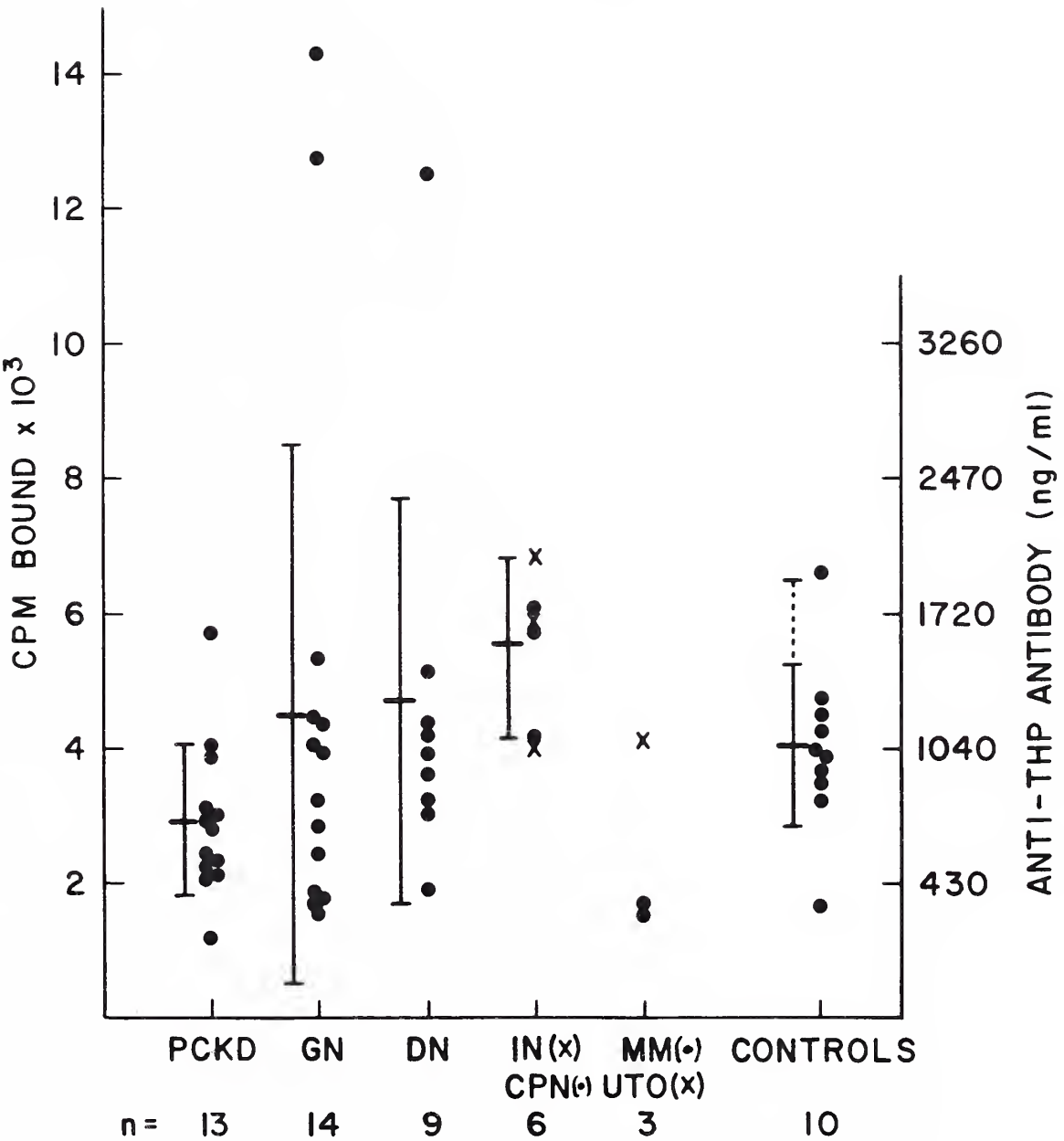

CPM BOUND x 10³
14
12
10
8
6
4
2
3260
2470
1720
1040
430
ANTI-THP ANTIBODY (ng/ml)
PCKD
GN
DN
IN(x) CPN(•)
MM(•) UTO(x)
CONTROLS
n = 13
14
9
6
3
10

FIGURE 2: Measurement of antibody to Tamm-Horsfall Protein (THP) in cpm and ng/ml in sera from patients with end-stage kidney disease who have significant bacterial counts in their urine (10^5 organisms per cc) of E. coli; other organisms including Enterobacter, Enterococcus, and diphtheroids; patients with sterile urine; patients who are anuric; and for comparison, non-infected controls.

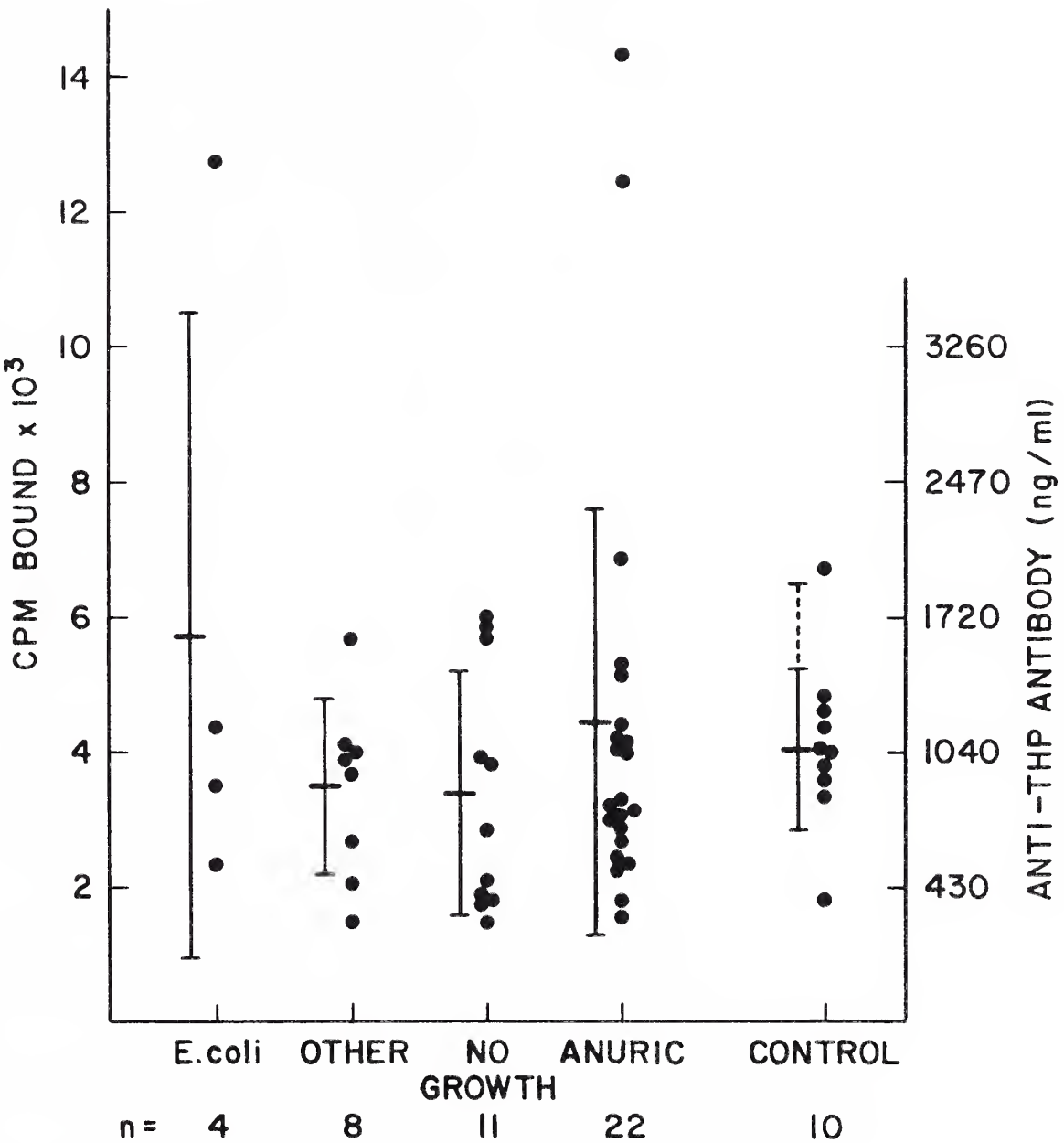

CPM BOUND x 10^3
14
12
10
8
6
4
2
3260
2470
1720
1040
430
ANTI-THP ANTIBODY (ng/ml)
E.coli
OTHER
NO GROWTH
ANURIC
CONTROL
n= 4
8
11
22
10

FIGURE 3: Measurement of antibody to Tamm-Horsfall Protein (THP) in sera from patients with end-stage kidney disease , represented as a function of number of months on dialysis.

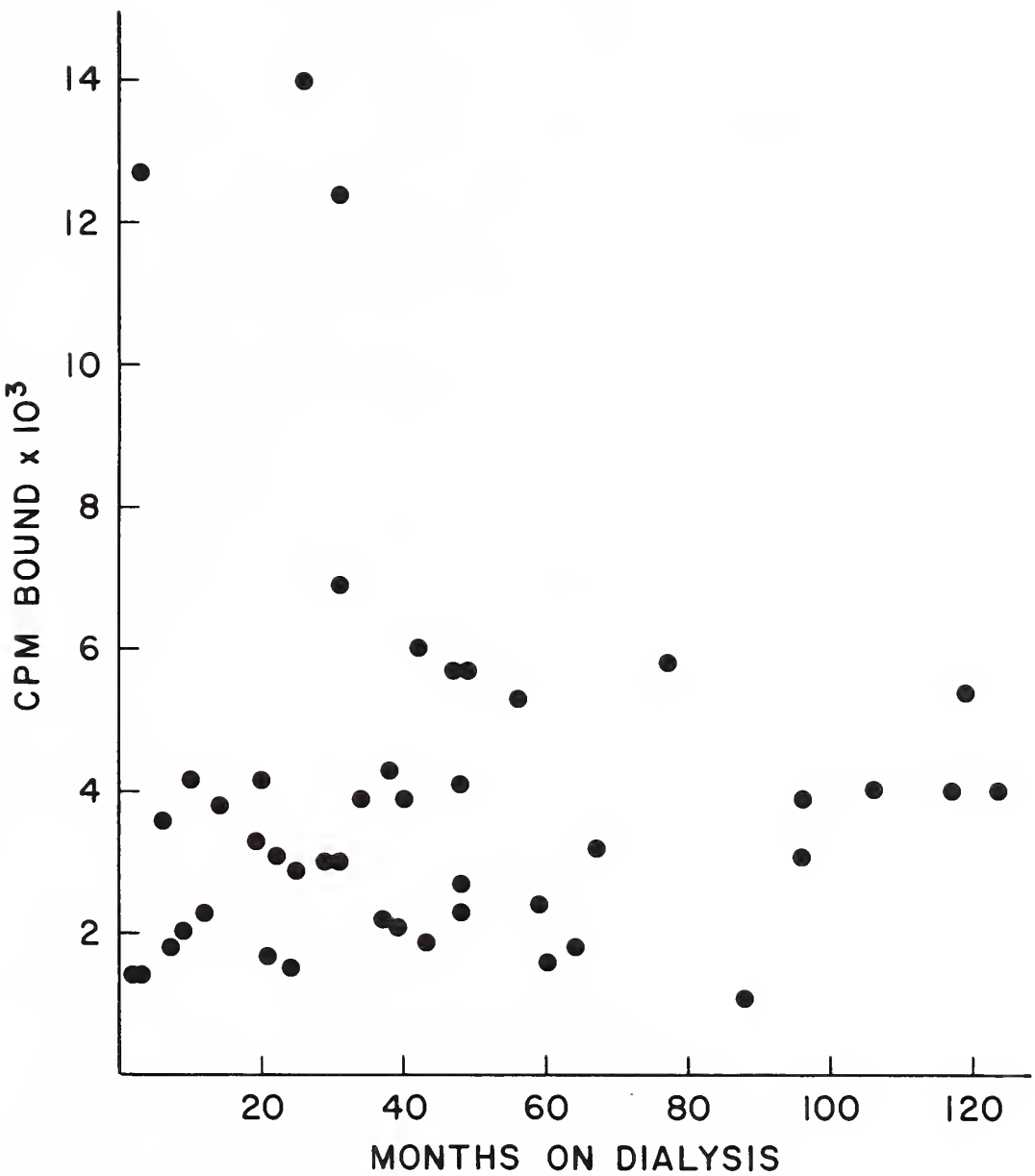

14
12
10
8
6
4
2
CPM BOUND x 10^3
20
40
60
80
100
120
MONTHS ON DIALYSIS

FIGURE 4: Measurement of antibody to Tamm-Horsfall Protein (THP) in sera from patients with polycystic kidney disease (PCKD) represented as a function of number of months on dialysis.

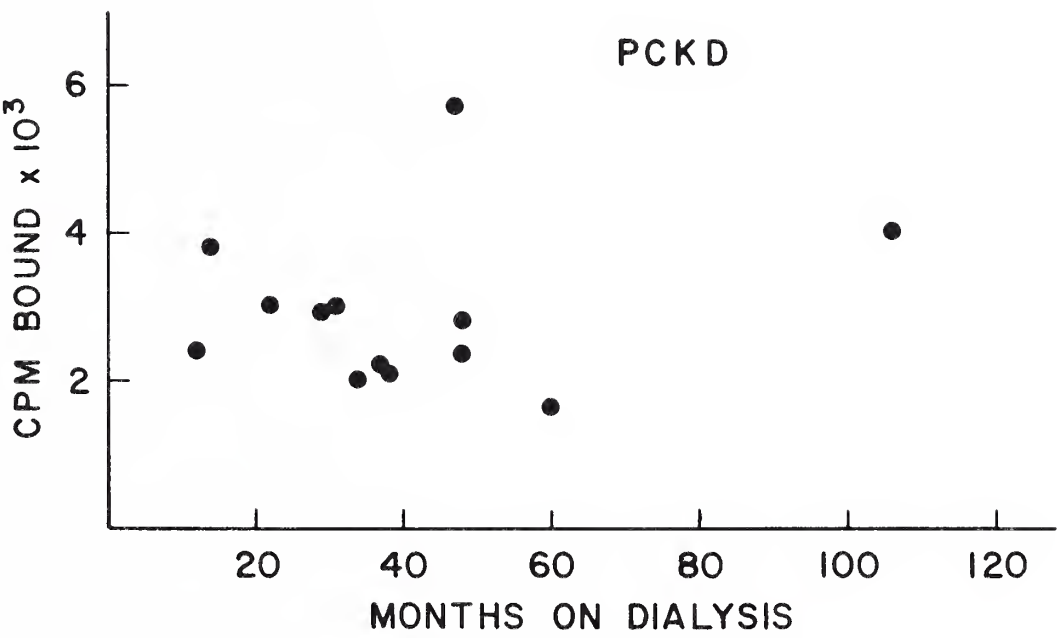

PCKD
CPM BOUND x 10^3
6
4
2
20
40
60
80
100
120
MONTHS ON DIALYSIS

FIGURE 5: Measurement of antibody to Tamm-Horsfall Protein (THP) in sera from patients with diabetic nephropathy (DN) expressed as a function of number of months on dialysis.

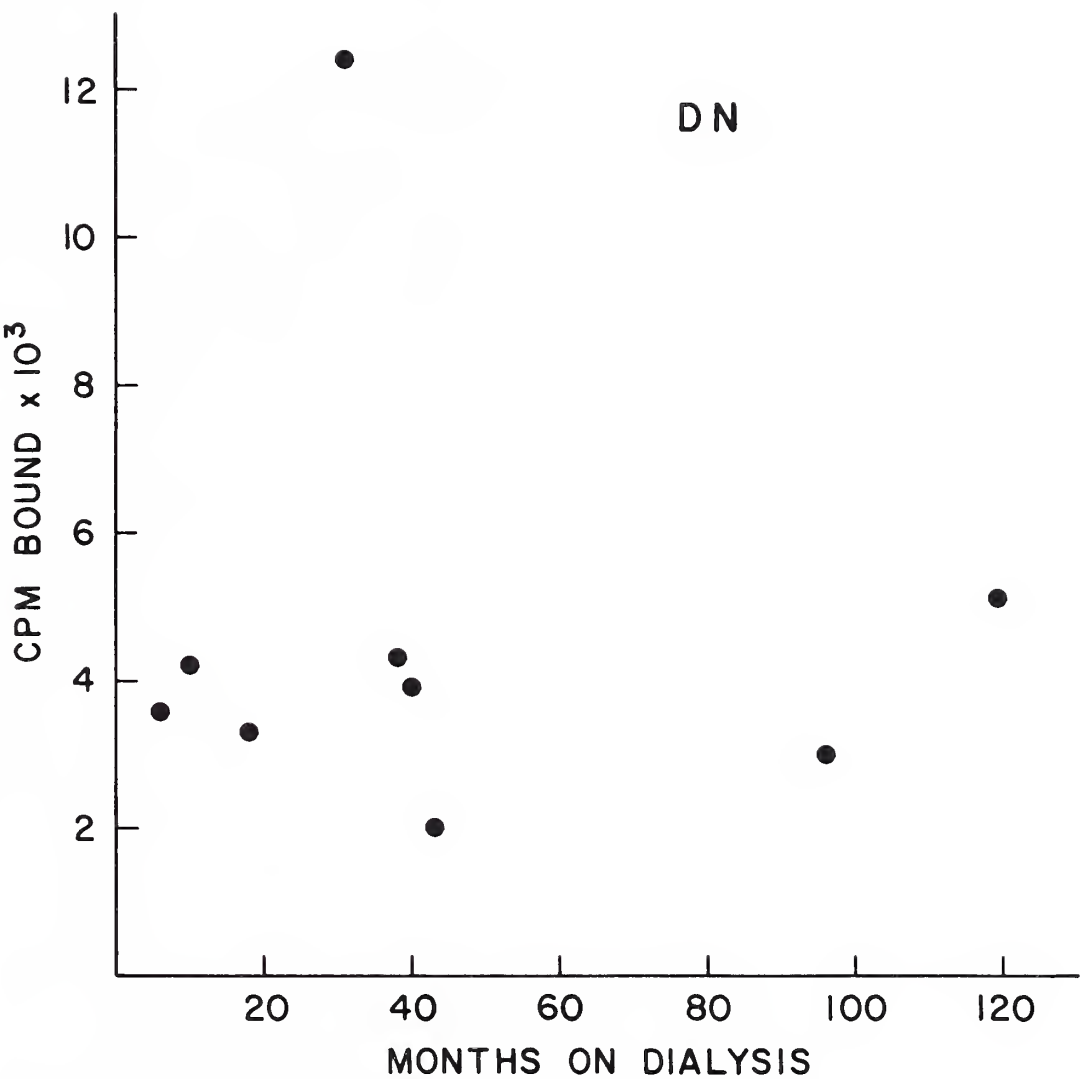

DN
CPM BOUND x 10^3
12
10
8
6
4
2
20
40
60
80
100
120
MONTHS ON DIALYSIS

FIGURE 6: Measurement of antibody to Tamm-Horsfall Protein (THP) in sera from patients with glomerulonephritis (GN) represented as a function of number of months on dialysis.

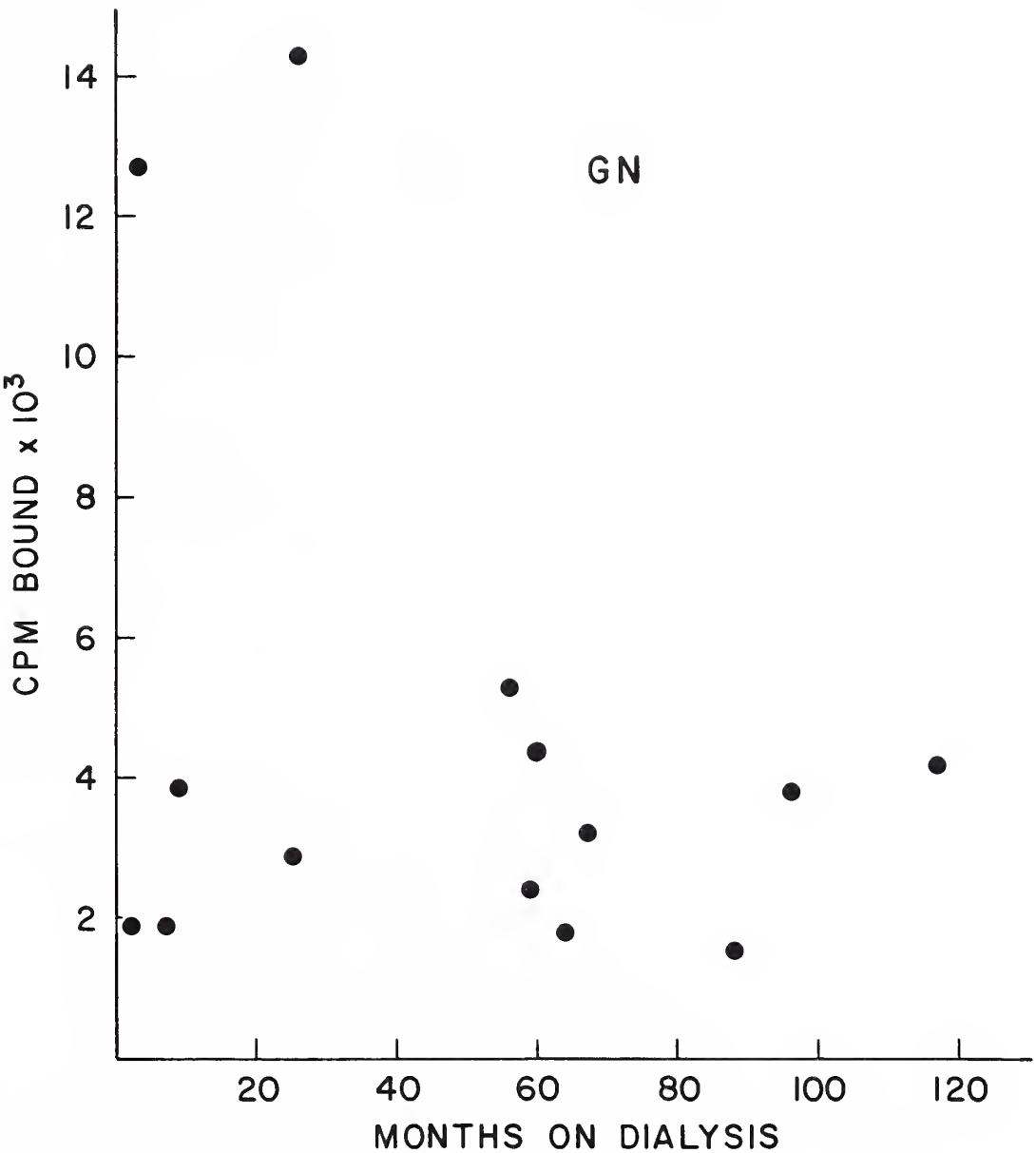

GN
CPM BOUND x 10^3
14
12
10
8
6
4
2
20
40
60
80
100
120
MONTHS ON DIALYSIS

FIGURE 7: Measurement of antibody to Tamm-Horsfall Protein (THP) in sera from patients with chronic Phelonephritis (CPN) and interstitial nephritis (IN) represented as a function of number of months on dialysis.

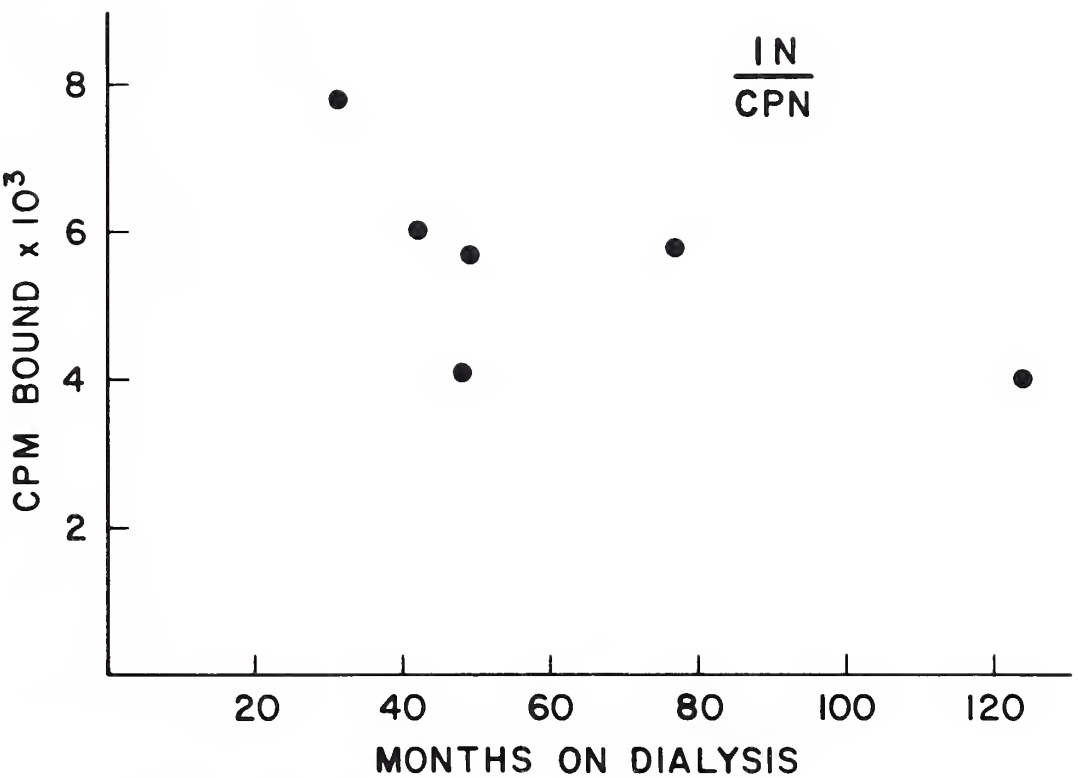

IN
CPN
CPM BOUND x 10^3
8
6
4
2
20
40
60
80
100
120
MONTHS ON DIALYSIS

FIGURE 8: Measurement of antibody to Tamm-Horsfall Protein (THP) in non-heparinized and heparinized samples obtained from volunteers. Titers expressed in cpm. Bar graphs represent one standard deviation.

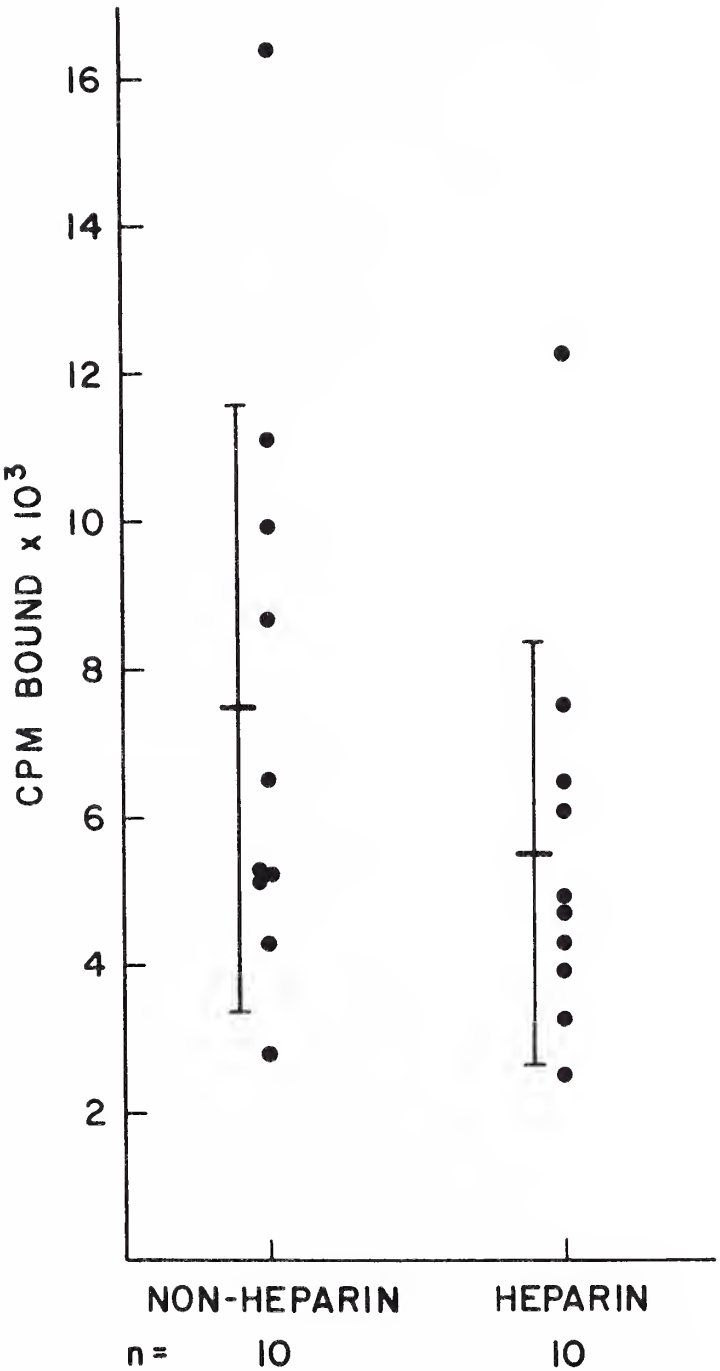
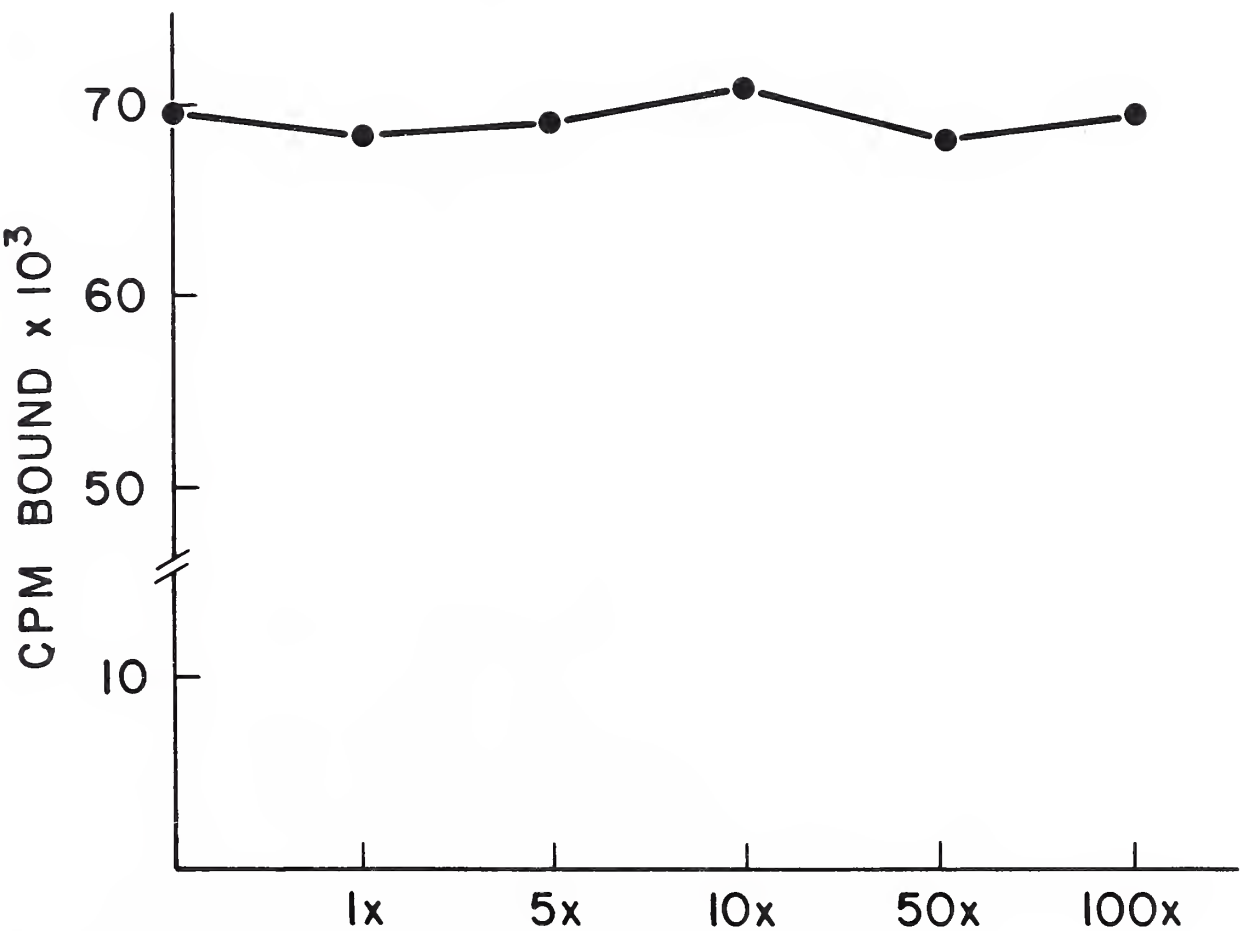

16
14
12
10
8
6
4
2
CPM BOUND x 10^3
NON-HEPARIN
HEPARIN
n = 10
10

FIGURE 9: Measurement of antibody to Tamm-Horsfall Protein (THP) in immune rabbit serum to which heparin was added to make final concentrations equal to, 5x, 10x, 50x, and 100x the concentration of heparin in patients at completion of dialysis.


70
60
50
10
CPM BOUND x 10^3
1x
5x
10x
50x
100x

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